

564462009640

## WHAT IS CLAIMED IS:

1. An isolated or recombinant nucleic acid comprising a nucleic acid sequence having at least 50% sequence identity to SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:131 or SEQ ID NO:133, over a region of at least about 100 residues, wherein the nucleic acid encodes at least one polypeptide having a pectate lyase activity, and the sequence identities are determined by analysis with a sequence comparison algorithm or by a visual inspection.
2. The isolated or recombinant nucleic acid of claim 1, wherein the sequence identity is at least about 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63% or 64%.
3. The isolated or recombinant nucleic acid of claim 1, wherein the sequence identity is at least about 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, or is 100% sequence identity to SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID

564462009640

NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID  
 NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID  
 NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID  
 NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID  
 5 NO:101, SEQ ID NO:103, SEQ ID NO:105, SEQ ID NO:107, SEQ ID NO:109, SEQ ID  
 NO:111, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID  
 NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID  
 NO:131 or SEQ ID NO:133.

10                   4.       The isolated or recombinant nucleic acid of claim 1, wherein the  
 sequence identity is over a region of at least about 50, 75, 100, 150, 200, 250, 300, 350,  
 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, 1100, 1150 or  
 more residues, or the full length of a gene or a transcript.

15                   5.       The isolated or recombinant nucleic acid of claim 1, wherein the  
 nucleic acid sequence comprises a sequence as set forth in SEQ ID NO:1, SEQ ID NO:3,  
 SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID  
 NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID  
 NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID  
 20 NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID  
 NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID  
 NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID  
 NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID  
 NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID  
 25 NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID  
 NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID  
 NO:105, SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ ID  
 NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:123, SEQ ID  
 NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:131 or SEQ ID NO:133.

30

                  6.       The isolated or recombinant nucleic acid of claim 1, wherein the  
 nucleic acid sequence encodes a polypeptide having a sequence as set forth in SEQ ID  
 NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12,  
 SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ

564462009640

ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:102, SEQ ID NO:104, SEQ ID NO:106, SEQ ID NO:108, SEQ ID NO:110, SEQ ID NO:112, SEQ ID NO:114, SEQ ID NO:116, SEQ ID NO:118, SEQ ID NO:120, SEQ ID NO:122, SEQ ID NO:124, SEQ ID NO:126, SEQ ID NO:128, SEQ ID NO:130, SEQ ID NO:132 or SEQ ID NO:134.

7. The isolated or recombinant nucleic acid of claim 1, wherein the sequence comparison algorithm is a BLAST version 2.2.2 algorithm where a filtering setting is set to blastall -p blastp -d "nr pataa" -F F, and all other options are set to default.

8. The isolated or recombinant nucleic acid of claim 1, wherein the pectate lyase activity comprises catalysis of beta-elimination (trans-elimination) or hydrolysis of pectin or polygalacturonic acid (pectate).

9. The isolated or recombinant nucleic acid of claim 8, wherein the pectate lyase activity comprises the breakup or dissolution of plant cell walls.

25

10. The isolated or recombinant nucleic acid of claim 1, wherein the pectate lyase activity comprises beta-elimination (trans-elimination) or hydrolysis of 1,4-linked alpha-D-galacturonic acid.

11. The isolated or recombinant nucleic acid of claim 9, wherein the pectate lyase activity comprises catalysis of beta-elimination (trans-elimination) or hydrolysis of methyl-esterified galacturonic acid.

564462009640

12. The isolated or recombinant nucleic acid of claim 1, wherein the pectate lyase activity is exo-acting or endo-acting.

13. The isolated or recombinant nucleic acid of claim 12, wherein the pectate lyase activity is endo-acting and acts at random sites within a polymer chain to give a mixture of oligomers.

14. The isolated or recombinant nucleic acid of claim 12, wherein the pectate lyase activity is exo-acting and acts from one end of a polymer chain and produces monomers or dimers.

15. The isolated or recombinant nucleic acid of claim 1, wherein the pectate lyase activity catalyzes the random cleavage of alpha-1,4-glycosidic linkages in pectic acid (polygalacturonic acid) by trans-elimination or hydrolysis.

16. The isolated or recombinant nucleic acid of claim 1, wherein the pectate lyase activity comprises activity the same or similar to pectate lyase (EC 4.2.2.2), poly(1,4-alpha-D-galacturonide) lyase, polygalacturonate lyase (EC 4.2.2.2), pectin lyase (EC 4.2.2.10), polygalacturonase (EC 3.2.1.15), exo-polygalacturonase (EC 3.2.1.67), exo-polygalacturonate lyase (EC 4.2.2.9) or exo-poly-alpha-galacturonosidase (EC 3.2.1.82).

17. The isolated or recombinant nucleic acid of claim 1, wherein the pectate lyase activity comprises beta-elimination (trans-elimination) or hydrolysis of galactan to galactose or galactooligomers.

18. The isolated or recombinant nucleic acid of claim 1, wherein the pectate lyase activity comprises beta-elimination (trans-elimination) or hydrolysis of a plant fiber.

19. The isolated or recombinant nucleic acid of claim 18, wherein the plant fiber comprises cotton fiber, hemp fiber or flax fiber.

564462009640

20. The isolated or recombinant nucleic acid of claim 1, wherein the pectate lyase activity is thermostable.

21. The isolated or recombinant nucleic acid of claim 20, wherein the polypeptide retains a pectate lyase activity under conditions comprising a temperature range of between about 37°C to about 95°C, or between about 55°C to about 85°C, or between about 70°C to about 75°C, or between about 70°C to about 95°C, or between about 90°C to about 95°C.

22. The isolated or recombinant nucleic acid of claim 1, wherein the pectate lyase activity is thermotolerant.

23. The isolated or recombinant nucleic acid of claim 22, wherein the polypeptide retains a pectate lyase activity after exposure to a temperature in the range from greater than 37°C to about 95°C, from greater than 55°C to about 85°C, or between about 70°C to about 75°C, or from greater than 90°C to about 95°C.

24. An isolated or recombinant nucleic acid, wherein the nucleic acid comprises a sequence that hybridizes under stringent conditions to a nucleic acid comprising SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:131 or SEQ ID NO:133, wherein the nucleic acid encodes a polypeptide having a pectate lyase activity.

564462009640

25. The isolated or recombinant nucleic acid of claim 24, wherein the nucleic acid is at least about 50, 75, 100, 150, 200, 300, 400, 500, 600, 700, 800, 900, 1000 or more residues in length or the full length of the gene or transcript.

5

26. The isolated or recombinant nucleic acid of claim 24, wherein the stringent conditions include a wash step comprising a wash in 0.2X SSC at a temperature of about 65°C for about 15 minutes.

10

27. A nucleic acid probe for identifying a nucleic acid encoding a polypeptide with a pectate lyase activity, wherein the probe comprises at least 10 consecutive bases of a sequence comprising SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:131 or SEQ ID NO:133, wherein the probe identifies the nucleic acid by binding or hybridization.

15

20

25

30

28. The nucleic acid probe of claim 27, wherein the probe comprises an oligonucleotide comprising at least about 10 to 50, about 20 to 60, about 30 to 70, about 40 to 80, about 60 to 100, or about 50 to 150 consecutive bases.

29. A nucleic acid probe for identifying a nucleic acid encoding a polypeptide having a pectate lyase activity, wherein the probe comprises a nucleic acid comprising at least about 10 consecutive residues of SEQ ID NO:1, SEQ ID NO:3, SEQ

564462009640

ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:131 or SEQ ID NO:133, wherein the sequence identities are determined by analysis with a sequence comparison algorithm or by visual inspection.

30. The nucleic acid probe of claim 29, wherein the probe comprises an oligonucleotide comprising at least about 10 to 50, about 20 to 60, about 30 to 70, about 40 to 80, about 60 to 100, or about 50 to 150 consecutive bases.

20

31. An amplification primer sequence pair for amplifying a nucleic acid encoding a polypeptide having a pectate lyase activity, wherein the primer pair is capable of amplifying a nucleic acid comprising a sequence as set forth in claim 1 or claim 24, or a subsequence thereof.

25

32. The amplification primer pair of claim 29, wherein each member of the amplification primer sequence pair comprises an oligonucleotide comprising at least about 10 to 50 consecutive bases of the sequence.

30

33. An amplification primer pair, wherein the primer pair comprises a first member having a sequence as set forth by about the first (the 5') 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 residues of SEQ ID NO:1 or SEQ ID NO:7, and a second member having a sequence as set forth by about the first (the 5') 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 residues of the complementary strand of the first member.

564462009640

34. A method of amplifying a nucleic acid encoding a polypeptide having a pectate lyase activity comprising amplification of a template nucleic acid with an amplification primer sequence pair capable of amplifying a nucleic acid sequence as set forth in claim 1 or claim 24, or a subsequence thereof.

35. An expression cassette comprising a nucleic acid comprising a sequence as set forth in claim 1 or claim 24.

36. A vector comprising a nucleic acid comprising a sequence as set forth in claim 1 or claim 24.

37. A cloning vehicle comprising a nucleic acid comprising a sequence as set forth in claim 1 or claim 24, wherein the cloning vehicle comprises a viral vector, a plasmid, a phage, a phagemid, a cosmid, a fosmid, a bacteriophage or an artificial chromosome.

38. The cloning vehicle of claim 37, wherein the viral vector comprises an adenovirus vector, a retroviral vector or an adeno-associated viral vector.

39. The cloning vehicle of claim 37, comprising a bacterial artificial chromosome (BAC), a plasmid, a bacteriophage P1-derived vector (PAC), a yeast artificial chromosome (YAC), or a mammalian artificial chromosome (MAC).

40. A transformed cell comprising a nucleic acid comprising a sequence as set forth in claim 1 or claim 24.

41. A transformed cell comprising an expression cassette as set forth in claim 35.

42. The transformed cell of claim 40, wherein the cell is a bacterial cell, a mammalian cell, a fungal cell, a yeast cell, an insect cell or a plant cell.



564462009640

43. A transgenic non-human animal comprising a sequence as set forth in claim 1 or claim 24.

44. The transgenic non-human animal of claim 43, wherein the animal  
5 is a mouse.

45. A transgenic plant comprising a sequence as set forth in claim 1 or claim 24.

46. The transgenic plant of claim 45, wherein the plant is a corn plant,  
10 a sorghum plant, a potato plant, a tomato plant, a wheat plant, an oilseed plant, a rapeseed plant, a soybean plant, a rice plant, a barley plant, a grass, or a tobacco plant.

47. A transgenic seed comprising a sequence as set forth in claim 1 or  
15 claim 24.

48. The transgenic seed of claim 47, wherein the seed is a corn seed, a wheat kernel, an oilseed, a rapeseed, a soybean seed, a palm kernel, a sunflower seed, a sesame seed, a rice, a barley, a peanut or a tobacco plant seed.

49. An antisense oligonucleotide comprising a nucleic acid sequence  
20 complementary to or capable of hybridizing under stringent conditions to a sequence as set forth in claim 1 or claim 24, or a subsequence thereof.

50. The antisense oligonucleotide of claim 49, wherein the antisense oligonucleotide is between about 10 to 50, about 20 to 60, about 30 to 70, about 40 to 80, or about 60 to 100 bases in length.

51. A method of inhibiting the translation of a pectate lyase message in  
30 a cell comprising administering to the cell or expressing in the cell an antisense oligonucleotide comprising a nucleic acid sequence complementary to or capable of hybridizing under stringent conditions to a sequence as set forth in claim 1 or claim 24.

564462009640

52. A double-stranded inhibitory RNA (RNAi) molecule comprising a subsequence of a sequence as set forth in claim 1 or claim 24.

53. The double-stranded inhibitory RNA (RNAi) molecule of claim 52,  
5 wherein the RNAi is about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25 or more duplex nucleotides in length.

54. A method of inhibiting the expression of a pectate lyase in a cell comprising administering to the cell or expressing in the cell a double-stranded inhibitory  
10 RNA (iRNA), wherein the RNA comprises a subsequence of a sequence as set forth in claim 1 or claim 24.

55. An isolated or recombinant polypeptide (i) having at least 50% sequence identity to SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID  
15 NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID  
20 NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:102, SEQ ID NO:104, SEQ ID NO:106, SEQ ID NO:108, SEQ ID  
25 NO:110, SEQ ID NO:112, SEQ ID NO:114, SEQ ID NO:116, SEQ ID NO:118, SEQ ID NO:120, SEQ ID NO:122, SEQ ID NO:124, SEQ ID NO:126, SEQ ID NO:128, SEQ ID NO:130, SEQ ID NO:132 or SEQ ID NO:134, over a region of at least about 100 residues, wherein the sequence identities are determined by analysis with a sequence comparison algorithm or by a visual inspection, or, (ii) encoded by a nucleic acid having  
30 at least 50% sequence identity to a sequence as set forth in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID

564462009640

NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:131 or SEQ ID NO:133 over a region of at least about 100 residues, and the sequence identities are determined by analysis with a sequence comparison algorithm or by a visual inspection, or encoded by a nucleic acid capable of hybridizing under stringent conditions to a sequence as set forth in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129 or SEQ ID NO:131, SEQ ID NO:133.

56. The isolated or recombinant polypeptide of claim 55, wherein the sequence identity is over a region of at least about at least about 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, or is 100% sequence identity.

564462009640

57. The isolated or recombinant polypeptide of claim 55, wherein the sequence identity is over a region of at least about 10, 15, 20, 25, 30, 35, 40, 45, 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050 or more residues, or the full length of an enzyme.

5

58. The isolated or recombinant polypeptide of claim 55, wherein the polypeptide has a sequence as set forth in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:102, SEQ ID NO:104, SEQ ID NO:106, SEQ ID NO:108, SEQ ID NO:110, SEQ ID NO:112, SEQ ID NO:114, SEQ ID NO:116, SEQ ID NO:118, SEQ ID NO:120, SEQ ID NO:122, SEQ ID NO:124, SEQ ID NO:126, SEQ ID NO:128, SEQ ID NO:130, SEQ ID NO:132 or SEQ ID NO:134.

20

59. The isolated or recombinant polypeptide of claim 55, wherein the polypeptide has a pectate lyase activity.

25

60. The isolated or recombinant polypeptide of claim 59, wherein the pectate lyase activity comprises catalysis of beta-elimination (trans-elimination) or hydrolysis of pectin or polygalacturonic acid (pectate).

30

61. The isolated or recombinant polypeptide of claim 60, wherein the pectate lyase activity comprises the breakup or dissolution of plant cell walls.

62. The isolated or recombinant polypeptide of claim 59, wherein the pectate lyase activity comprises beta-elimination (trans-elimination) or hydrolysis of 1,4-linked alpha-D-galacturonic acid.

564462009640

63. The isolated or recombinant nucleic acid of polypeptide of claim 59, wherein the pectate lyase activity comprises catalysis of beta-elimination (trans-elimination) or hydrolysis of methyl-esterified galacturonic acid.
- 5 64. The isolated or recombinant polypeptide of claim 59, wherein the pectate lyase activity is exo-acting or endo-acting.
65. The isolated or recombinant polypeptide of claim 64, wherein the  
10 pectate lyase activity is endo-acting and acts at random sites within a polymer chain to give a mixture of oligomers.
66. The isolated or recombinant polypeptide of claim 64, wherein the  
15 pectate lyase activity is exo-acting and acts from one end of a polymer chain and produces monomers or dimers.
67. The isolated or recombinant polypeptide of claim 59, wherein the  
20 pectate lyase activity catalyzes the random cleavage of alpha-1,4-glycosidic linkages in pectic acid (polygalacturonic acid) by trans-elimination or hydrolysis.
68. The isolated or recombinant polypeptide of claim 59, wherein the  
pectate lyase activity comprises activity the same or similar to pectate lyase (EC 4.2.2.2),  
poly(1,4-alpha-D-galacturonide) lyase, polygalacturonate lyase (EC 4.2.2.2), pectin lyase  
(EC 4.2.2.10), polygalacturonase (EC 3.2.1.15), exo-polygalacturonase (EC 3.2.1.67),  
25 exo-polygalacturonate lyase (EC 4.2.2.9) or exo-poly-alpha-galacturonosidase (EC  
3.2.1.82).
69. The isolated or recombinant polypeptide of claim 59, wherein the  
pectate lyase activity comprises beta-elimination (trans-elimination) or hydrolysis of  
30 galactan to galactose or galactooligomers.
70. The isolated or recombinant polypeptide of claim 59, wherein the  
pectate lyase activity comprises beta-elimination (trans-elimination) or hydrolysis of a  
plant fiber.

564462009640

71. The isolated or recombinant polypeptide of claim 59, wherein the plant fiber comprises cotton fiber, hemp fiber or flax fiber.

5 72. The isolated or recombinant polypeptide of claim 59, wherein the pectate lyase activity is thermostable.

73. The isolated or recombinant polypeptide of claim 72, wherein the polypeptide retains a pectate lyase activity under conditions comprising a temperature  
10 range of between about 37°C to about 95°C, between about 55°C to about 85°C, between about 70°C to about 95°C, between about 70°C to about 75°C, or between about 90°C to about 95°C.

74. The isolated or recombinant polypeptide of claim 59, wherein the  
15 pectate lyase activity is thermotolerant.

75. The isolated or recombinant polypeptide of claim 74, wherein the polypeptide retains a pectate lyase activity after exposure to a temperature in the range from greater than 37°C to about 95°C, from greater than 55°C to about 85°C, between  
20 about 70°C to about 75°C, or from greater than 90°C to about 95°C.

76. An isolated or recombinant polypeptide comprising a polypeptide as set forth in claim 55 and lacking a signal sequence.

25 77. An isolated or recombinant polypeptide comprising a polypeptide as set forth in claim 55 and having a heterologous signal sequence.

78. The isolated or recombinant polypeptide of claim 59, wherein the pectate lyase activity comprises a specific activity at about 37°C in the range from about  
30 100 to about 1000 units per milligram of protein, from about 500 to about 750 units per milligram of protein, from about 500 to about 1200 units per milligram of protein, or from about 750 to about 1000 units per milligram of protein.

564462009640

79. The isolated or recombinant polypeptide of claim 74, wherein the thermotolerance comprises retention of at least half of the specific activity of the pectate lyase at 37°C after being heated to an elevated temperature.

5 80. The isolated or recombinant polypeptide of claim 74, wherein the thermotolerance comprises retention of specific activity at 37°C in the range from about 500 to about 1200 units per milligram of protein after being heated to an elevated temperature.

10 81. The isolated or recombinant polypeptide of claim 55, wherein the polypeptide comprises at least one glycosylation site.

82. The isolated or recombinant polypeptide of claim 81, wherein the glycosylation is an N-linked glycosylation.

15 83. The isolated or recombinant polypeptide of claim 82, wherein the polypeptide is glycosylated after being expressed in a *P. pastoris* or a *S. pombe*.

84. The isolated or recombinant polypeptide of claim 59, wherein the polypeptide retains a pectate lyase activity under conditions comprising about pH 6.5, pH 6.0, pH 5.5, 5.0, pH 4.5 or 4.0.

20 85. The isolated or recombinant polypeptide of claim 59, wherein the polypeptide retains a pectate lyase activity under conditions comprising about pH 7.5, pH 8.0, pH 8.5, pH 9, pH 9.5, pH 10 or pH 10.5.

86. A protein preparation comprising a polypeptide as set forth in claim 55, wherein the protein preparation comprises a liquid, a solid or a gel.

30 87. A heterodimer comprising a polypeptide as set forth in claim 55 and a second domain.

88. The heterodimer of claim 87, wherein the second domain is a polypeptide and the heterodimer is a fusion protein.

564462009640

89. The heterodimer of claim 88, wherein the second domain is an epitope or a tag.
- 5 90. A homodimer comprising a polypeptide as set forth in claim 55.
91. An immobilized polypeptide, wherein the polypeptide comprises a sequence as set forth in claim 55, or a subsequence thereof.
- 10 92. The immobilized polypeptide of claim 91, wherein the polypeptide is immobilized on a cell, a metal, a resin, a polymer, a ceramic, a glass, a microelectrode, a graphitic particle, a bead, a gel, a plate, an array or a capillary tube.
- 15 93. An array comprising an immobilized polypeptide as set forth in claim 55.
94. An array comprising an immobilized nucleic acid as set forth in claim 1 or claim 24.
- 20 95. An isolated or recombinant antibody that specifically binds to a polypeptide as set forth in claim 55.
96. The isolated or recombinant antibody of claim 95, wherein the antibody is a monoclonal or a polyclonal antibody.
- 25 97. A hybridoma comprising an antibody that specifically binds to a polypeptide as set forth in claim 55.
98. A method of isolating or identifying a polypeptide with a pectate  
30 lyase activity comprising the steps of:  
(a) providing an antibody as set forth in claim 95;  
(b) providing a sample comprising polypeptides; and



564462009640

(c) contacting the sample of step (b) with the antibody of step (a) under conditions wherein the antibody can specifically bind to the polypeptide, thereby isolating or identifying a polypeptide having a pectate lyase activity.

5                    99.     A method of making an anti-pectate lyase antibody comprising administering to a non-human animal a nucleic acid as set forth in claim 1 or claim 24 or a subsequence thereof in an amount sufficient to generate a humoral immune response, thereby making an anti-pectate lyase antibody.

10                   100.    A method of making an anti-pectate lyase antibody comprising administering to a non-human animal a polypeptide as set forth in claim 55 or a subsequence thereof in an amount sufficient to generate a humoral immune response, thereby making an anti-pectate lyase antibody.

15                   101.    A method of producing a recombinant polypeptide comprising the steps of: (a) providing a nucleic acid operably linked to a promoter, wherein the nucleic acid comprises a sequence as set forth in claim 1 or claim 24; and (b) expressing the nucleic acid of step (a) under conditions that allow expression of the polypeptide, thereby producing a recombinant polypeptide.

20                   102.    The method of claim 106, further comprising transforming a host cell with the nucleic acid of step (a) followed by expressing the nucleic acid of step (a), thereby producing a recombinant polypeptide in a transformed cell.

25                   103.    A method for identifying a polypeptide having a pectate lyase activity comprising the following steps:  
                          (a) providing a polypeptide as set forth in claim 59;  
                          (b) providing a pectate lyase substrate; and  
                          (c) contacting the polypeptide with the substrate of step (b) and detecting a  
 30    decrease in the amount of substrate or an increase in the amount of a reaction product, wherein a decrease in the amount of the substrate or an increase in the amount of the reaction product detects a polypeptide having a pectate lyase activity.

564462009640

104. A method for identifying a pectate lyase substrate comprising the following steps:

- (a) providing a polypeptide as set forth in claim 59;
- (b) providing a test substrate; and
- 5 (c) contacting the polypeptide of step (a) with the test substrate of step (b) and detecting a decrease in the amount of substrate or an increase in the amount of reaction product, wherein a decrease in the amount of the substrate or an increase in the amount of a reaction product identifies the test substrate as a pectate lyase substrate.

10 105. A method of determining whether a test compound specifically binds to a polypeptide comprising the following steps:

- (a) expressing a nucleic acid or a vector comprising the nucleic acid under conditions permissive for translation of the nucleic acid to a polypeptide, wherein the nucleic acid has a sequence as set forth in claim 1 or claim 24;
- 15 (b) providing a test compound;
- (c) contacting the polypeptide with the test compound; and
- (d) determining whether the test compound of step (b) specifically binds to the polypeptide.

20 106. A method of determining whether a test compound specifically binds to a polypeptide comprising the following steps:

- (a) providing a polypeptide as set forth in claim 55;
- (b) providing a test compound;
- (c) contacting the polypeptide with the test compound; and
- 25 (d) determining whether the test compound of step (b) specifically binds to the polypeptide.

107. A method for identifying a modulator of a pectate lyase activity comprising the following steps:

- 30 (a) providing a polypeptide as set forth in claim 59;
- (b) providing a test compound;
- (c) contacting the polypeptide of step (a) with the test compound of step (b) and measuring an activity of the pectate lyase, wherein a change in the pectate lyase activity measured in the presence of the test compound compared to the activity in the

564462009640

absence of the test compound provides a determination that the test compound modulates the pectate lyase activity.

108. The method of claim 107, wherein the pectate lyase activity is  
5 measured by providing a pectate lyase substrate and detecting a decrease in the amount of the substrate or an increase in the amount of a reaction product, or, an increase in the amount of the substrate or a decrease in the amount of a reaction product.

109. The method of claim 107, wherein a decrease in the amount of the  
10 substrate or an increase in the amount of the reaction product with the test compound as compared to the amount of substrate or reaction product without the test compound identifies the test compound as an activator of pectate lyase activity.

110. The method of claim 107, wherein an increase in the amount of the  
15 substrate or a decrease in the amount of the reaction product with the test compound as compared to the amount of substrate or reaction product without the test compound identifies the test compound as an inhibitor of pectate lyase activity.

111. A computer system comprising a processor and a data storage  
20 device wherein said data storage device has stored thereon a polypeptide sequence or a nucleic acid sequence, wherein the polypeptide sequence comprises sequence as set forth in claim 55, a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24.

112. The computer system of claim 111, further comprising a sequence  
25 comparison algorithm and a data storage device having at least one reference sequence stored thereon.

113. The computer system of claim 112, wherein the sequence  
comparison algorithm comprises a computer program that indicates polymorphisms.

30

114. The computer system of claim 112, further comprising an identifier  
that identifies one or more features in said sequence.

564462009640

115. A computer readable medium having stored thereon a polypeptide sequence or a nucleic acid sequence, wherein the polypeptide sequence comprises a polypeptide as set forth in claim 55; a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24.

5

116. A method for identifying a feature in a sequence comprising the steps of: (a) reading the sequence using a computer program which identifies one or more features in a sequence, wherein the sequence comprises a polypeptide sequence or a nucleic acid sequence, wherein the polypeptide sequence comprises a polypeptide as set forth in claim 55; a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24; and (b) identifying one or more features in the sequence with the computer program.

10

117. A method for comparing a first sequence to a second sequence comprising the steps of: (a) reading the first sequence and the second sequence through use of a computer program which compares sequences, wherein the first sequence comprises a polypeptide sequence or a nucleic acid sequence, wherein the polypeptide sequence comprises a polypeptide as set forth in claim 55 or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24; and (b) determining differences between the first sequence and the second sequence with the computer program.

15  
20

118. The method of claim 117, wherein the step of determining differences between the first sequence and the second sequence further comprises the step of identifying polymorphisms.

25

119. The method of claim 117, further comprising an identifier that identifies one or more features in a sequence.

120. The method of claim 119, comprising reading the first sequence using a computer program and identifying one or more features in the sequence.

30

121. A method for isolating or recovering a nucleic acid encoding a polypeptide with a pectate lyase activity from an environmental sample comprising the steps of:

564462009640

(a) providing an amplification primer sequence pair as set forth in claim 31 or claim 33;

(b) isolating a nucleic acid from the environmental sample or treating the environmental sample such that nucleic acid in the sample is accessible for hybridization to the amplification primer pair; and,

(c) combining the nucleic acid of step (b) with the amplification primer pair of step (a) and amplifying nucleic acid from the environmental sample, thereby isolating or recovering a nucleic acid encoding a polypeptide with a pectate lyase activity from an environmental sample.

10

122. The method of claim 121, wherein each member of the amplification primer sequence pair comprises an oligonucleotide comprising at least about 10 to 50 consecutive bases of a sequence as set forth in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:131 or SEQ ID NO:133, or a subsequence thereof.

123. A method for isolating or recovering a nucleic acid encoding a polypeptide with a pectate lyase activity from an environmental sample comprising the steps of:

(a) providing a polynucleotide probe comprising a sequence as set forth in claim 1 or claim 24, or a subsequence thereof;

564462009640

(b) isolating a nucleic acid from the environmental sample or treating the environmental sample such that nucleic acid in the sample is accessible for hybridization to a polynucleotide probe of step (a);

(c) combining the isolated nucleic acid or the treated environmental sample of step (b) with the polynucleotide probe of step (a); and

(d) isolating a nucleic acid that specifically hybridizes with the polynucleotide probe of step (a), thereby isolating or recovering a nucleic acid encoding a polypeptide with a pectate lyase activity from an environmental sample.

124. The method of claim 121 or claim 123, wherein the environmental sample comprises a water sample, a liquid sample, a soil sample, an air sample or a biological sample.

125. The method of claim 124, wherein the biological sample is derived from a bacterial cell, a protozoan cell, an insect cell, a yeast cell, a plant cell, a fungal cell or a mammalian cell.

126. A method of generating a variant of a nucleic acid encoding a polypeptide with a pectate lyase activity comprising the steps of:

(a) providing a template nucleic acid comprising a sequence as set forth in claim 1 or claim 24; and

(b) modifying, deleting or adding one or more nucleotides in the template sequence, or a combination thereof, to generate a variant of the template nucleic acid.

127. The method of claim 126, further comprising expressing the variant nucleic acid to generate a variant pectate lyase polypeptide.

128. The method of claim 126, wherein the modifications, additions or deletions are introduced by a method comprising error-prone PCR, shuffling, oligonucleotide-directed mutagenesis, assembly PCR, sexual PCR mutagenesis, *in vivo* mutagenesis, cassette mutagenesis, recursive ensemble mutagenesis, exponential ensemble mutagenesis, site-specific mutagenesis, gene reassembly, gene site saturation mutagenesis (GSSM™), synthetic ligation reassembly (SLR) and a combination thereof.

564462009640

129. The method of claim 126, wherein the modifications, additions or deletions are introduced by a method comprising recombination, recursive sequence recombination, phosphothioate-modified DNA mutagenesis, uracil-containing template mutagenesis, gapped duplex mutagenesis, point mismatch repair mutagenesis, repair-  
5 deficient host strain mutagenesis, chemical mutagenesis, radiogenic mutagenesis, deletion mutagenesis, restriction-selection mutagenesis, restriction-purification mutagenesis, artificial gene synthesis, ensemble mutagenesis, chimeric nucleic acid multimer creation and a combination thereof.

10 130. The method of claim 126, wherein the method is iteratively repeated until a pectate lyase having an altered or different activity or an altered or different stability from that of a polypeptide encoded by the template nucleic acid is produced.

15 131. The method of claim 130, wherein the variant pectate lyase polypeptide is thermotolerant, and retains some activity after being exposed to an elevated temperature.

20 132. The method of claim 127, wherein the variant pectate lyase polypeptide has increased glycosylation as compared to the pectate lyase encoded by a template nucleic acid.

25 133. The method of claim 127, wherein the variant pectate lyase polypeptide has a pectate lyase activity under a high temperature, wherein the pectate lyase encoded by the template nucleic acid is not active under the high temperature.

30 134. The method of claim 126, wherein the method is iteratively repeated until a pectate lyase coding sequence having an altered codon usage from that of the template nucleic acid is produced.

135. The method of claim 126, wherein the method is iteratively repeated until a pectate lyase gene having higher or lower level of message expression or stability from that of the template nucleic acid is produced.

564462009640

136. A method for modifying codons in a nucleic acid encoding a polypeptide with a pectate lyase activity to increase its expression in a host cell, the method comprising the following steps:

- (a) providing a nucleic acid encoding a polypeptide with a pectate lyase activity comprising a sequence as set forth in claim 1 or claim 24; and,
- (b) identifying a non-preferred or a less preferred codon in the nucleic acid of step (a) and replacing it with a preferred or neutrally used codon encoding the same amino acid as the replaced codon, wherein a preferred codon is a codon over-represented in coding sequences in genes in the host cell and a non-preferred or less preferred codon is a codon under-represented in coding sequences in genes in the host cell, thereby modifying the nucleic acid to increase its expression in a host cell.

137. A method for modifying codons in a nucleic acid encoding a pectate lyase polypeptide, the method comprising the following steps:

- (a) providing a nucleic acid encoding a polypeptide with a pectate lyase activity comprising a sequence as set forth in claim 1 or claim 24; and,
- (b) identifying a codon in the nucleic acid of step (a) and replacing it with a different codon encoding the same amino acid as the replaced codon, thereby modifying codons in a nucleic acid encoding a pectate lyase.

138. A method for modifying codons in a nucleic acid encoding a pectate lyase polypeptide to increase its expression in a host cell, the method comprising the following steps:

- (a) providing a nucleic acid encoding a pectate lyase polypeptide comprising a sequence as set forth in claim 1 or claim 24; and,
- (b) identifying a non-preferred or a less preferred codon in the nucleic acid of step (a) and replacing it with a preferred or neutrally used codon encoding the same amino acid as the replaced codon, wherein a preferred codon is a codon over-represented in coding sequences in genes in the host cell and a non-preferred or less preferred codon is a codon under-represented in coding sequences in genes in the host cell, thereby modifying the nucleic acid to increase its expression in a host cell.



564462009640

139. A method for modifying a codon in a nucleic acid encoding a polypeptide having a pectate lyase activity to decrease its expression in a host cell, the method comprising the following steps:

- (a) providing a nucleic acid encoding a pectate lyase polypeptide  
5 comprising a sequence as set forth in claim 1 or claim 24; and
- (b) identifying at least one preferred codon in the nucleic acid of step (a) and replacing it with a non-preferred or less preferred codon encoding the same amino acid as the replaced codon, wherein a preferred codon is a codon over-represented in coding sequences in genes in a host cell and a non-preferred or less preferred codon is a  
10 codon under-represented in coding sequences in genes in the host cell, thereby modifying the nucleic acid to decrease its expression in a host cell.

140. The method of claim 138 or 139, wherein the host cell is a bacterial cell, a fungal cell, an insect cell, a yeast cell, a plant cell or a mammalian cell.

15

141. A method for producing a library of nucleic acids encoding a plurality of modified pectate lyase active sites or substrate binding sites, wherein the modified active sites or substrate binding sites are derived from a first nucleic acid comprising a sequence encoding a first active site or a first substrate binding site the  
20 method comprising the following steps:

- (a) providing a first nucleic acid encoding a first active site or first substrate binding site, wherein the first nucleic acid sequence comprises a sequence that hybridizes under stringent conditions to a sequence as set forth in SEQ ID NO:1 or SEQ ID NO:7, or a subsequence thereof, and the nucleic acid encodes a pectate lyase active  
25 site or a pectate lyase substrate binding site;

(b) providing a set of mutagenic oligonucleotides that encode naturally-occurring amino acid variants at a plurality of targeted codons in the first nucleic acid; and,

- (c) using the set of mutagenic oligonucleotides to generate a set of active  
30 site-encoding or substrate binding site-encoding variant nucleic acids encoding a range of amino acid variations at each amino acid codon that was mutagenized, thereby producing a library of nucleic acids encoding a plurality of modified pectate lyase active sites or substrate binding sites.

564462009640

142. The method of claim 141, comprising mutagenizing the first nucleic acid of step (a) by a method comprising an optimized directed evolution system, gene site-saturation mutagenesis (GSSM™), or a synthetic ligation reassembly (SLR).

5 143. The method of claim 141, comprising mutagenizing the first nucleic acid of step (a) or variants by a method comprising error-prone PCR, shuffling, oligonucleotide-directed mutagenesis, assembly PCR, sexual PCR mutagenesis, in vivo mutagenesis, cassette mutagenesis, recursive ensemble mutagenesis, exponential ensemble mutagenesis, site-specific mutagenesis, gene reassembly, gene site saturation  
10 mutagenesis (GSSM™), synthetic ligation reassembly (SLR) and a combination thereof.

144. The method of claim 141, comprising mutagenizing the first nucleic acid of step (a) or variants by a method comprising recombination, recursive sequence recombination, phosphothioate-modified DNA mutagenesis, uracil-containing  
15 template mutagenesis, gapped duplex mutagenesis, point mismatch repair mutagenesis, repair-deficient host strain mutagenesis, chemical mutagenesis, radiogenic mutagenesis, deletion mutagenesis, restriction-selection mutagenesis, restriction-purification mutagenesis, artificial gene synthesis, ensemble mutagenesis, chimeric nucleic acid multimer creation and a combination thereof.

20

145. A method for making a small molecule comprising the following steps:

(a) providing a plurality of biosynthetic enzymes capable of synthesizing or modifying a small molecule, wherein one of the enzymes comprises a pectate lyase  
25 enzyme encoded by a nucleic acid comprising a sequence as set forth in claim 1 or claim 24;

(b) providing a substrate for at least one of the enzymes of step (a); and  
(c) reacting the substrate of step (b) with the enzymes under conditions that facilitate a plurality of biocatalytic reactions to generate a small molecule by a series  
30 of biocatalytic reactions.

146. A method for modifying a small molecule comprising the following steps:

564462009640

(a) providing a pectate lyase enzyme, wherein the enzyme comprises a polypeptide as set forth in claim 59, or a polypeptide encoded by a nucleic acid comprising a nucleic acid sequence as set forth in claim 1 or claim 24;

(b) providing a small molecule; and

5 (c) reacting the enzyme of step (a) with the small molecule of step (b) under conditions that facilitate an enzymatic reaction catalyzed by the pectate lyase enzyme, thereby modifying a small molecule by a pectate lyase enzymatic reaction.

10 147. The method of claim 146, comprising a plurality of small molecule substrates for the enzyme of step (a), thereby generating a library of modified small molecules produced by at least one enzymatic reaction catalyzed by the pectate lyase enzyme.

15 148. The method of claim 146, further comprising a plurality of additional enzymes under conditions that facilitate a plurality of biocatalytic reactions by the enzymes to form a library of modified small molecules produced by the plurality of enzymatic reactions.

20 149. The method of claim 148, further comprising the step of testing the library to determine if a particular modified small molecule which exhibits a desired activity is present within the library.

25 150. The method of claim 149, wherein the step of testing the library further comprises the steps of systematically eliminating all but one of the biocatalytic reactions used to produce a portion of the plurality of the modified small molecules within the library by testing the portion of the modified small molecule for the presence or absence of the particular modified small molecule with a desired activity, and identifying at least one specific biocatalytic reaction that produces the particular modified small molecule of desired activity.

30

151. A method for determining a functional fragment of a pectate lyase enzyme comprising the steps of:

564462009640

(a) providing a pectate lyase enzyme, wherein the enzyme comprises a polypeptide as set forth in claim 59, or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24; and

(b) deleting a plurality of amino acid residues from the sequence of step

- 5 (a) and testing the remaining subsequence for a pectate lyase activity, thereby determining a functional fragment of a pectate lyase enzyme.

152. The method of claim 151, wherein the pectate lyase activity is measured by providing a pectate lyase substrate and detecting a decrease in the amount of  
10 the substrate or an increase in the amount of a reaction product.

153. A method for whole cell engineering of new or modified phenotypes by using real-time metabolic flux analysis, the method comprising the following steps:

- 15 (a) making a modified cell by modifying the genetic composition of a cell, wherein the genetic composition is modified by addition to the cell of a nucleic acid comprising a sequence as set forth in claim 1 or claim 24;

(b) culturing the modified cell to generate a plurality of modified cells;

- (c) measuring at least one metabolic parameter of the cell by monitoring  
20 the cell culture of step (b) in real time; and,

(d) analyzing the data of step (c) to determine if the measured parameter differs from a comparable measurement in an unmodified cell under similar conditions, thereby identifying an engineered phenotype in the cell using real-time metabolic flux analysis.

25

154. The method of claim 153, wherein the genetic composition of the cell is modified by a method comprising deletion of a sequence or modification of a sequence in the cell, or, knocking out the expression of a gene.

- 30 155. The method of claim 153, further comprising selecting a cell comprising a newly engineered phenotype.

156. The method of claim 155, further comprising culturing the selected cell, thereby generating a new cell strain comprising a newly engineered phenotype.

564462009640

157. An isolated or recombinant signal sequence consisting of a signal peptides (SP) as set forth in Table 1.

5 158. An isolated or recombinant signal sequence consisting of a sequence as set forth in residues 1 to 15, 1 to 16, 1 to 17, 1 to 18, 1 to 19, 1 to 20, 1 to 21, 1 to 22, 1 to 23, 1 to 24, 1 to 25, 1 to 26, 1 to 27, 1 to 28, 1 to 28, 1 to 30, 1 to 31, 1 to 32, 1 to 33, 1 to 34, 1 to 35, 1 to 36, 1 to 37, 1 to 38, 1 to 39, 1 to 40, 1 to 41, 1 to 42, 1 to 43 or 1 to 44, of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID  
10 NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID  
15 NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:102, SEQ ID NO:104, SEQ ID NO:106, SEQ ID NO:108, SEQ ID  
20 NO:110, SEQ ID NO:112, SEQ ID NO:114, SEQ ID NO:116, SEQ ID NO:118, SEQ ID NO:120, SEQ ID NO:122, SEQ ID NO:124, SEQ ID NO:126, SEQ ID NO:128 or SEQ ID NO:130, SEQ ID NO:132 or SEQ ID NO:134.

25 159. An isolated or recombinant peptide consisting of a pectin methyl esterase domain (PED) or a catalytic domain (CD) as set forth in Table 1.

160. An chimeric polypeptide comprising at least a first domain comprising signal peptide (SP), a pectin methyl esterase domain (PED) or a catalytic domain (CD) as set forth in Table 1 and at least a second domain comprising a  
30 heterologous polypeptide or peptide, wherein the heterologous polypeptide or peptide is not naturally associated with the signal peptide (SP), pectin methyl esterase domain (PED) or catalytic domain (CD).

564462009640

161. The chimeric polypeptide of claim 160, wherein the heterologous polypeptide or peptide is not a pectate lyase.

162. The chimeric polypeptide of claim 160, wherein the heterologous polypeptide or peptide is amino terminal to, carboxy terminal to or on both ends of the signal peptide (SP), pectin methyl esterase domain (PED) or catalytic domain (CD).

163. An isolated or recombinant nucleic acid encoding a chimeric polypeptide, wherein the chimeric polypeptide comprises at least a first domain comprising signal peptide (SP), a pectin methyl esterase domain (PED) or a catalytic domain (CD) as set forth in Table 1 and at least a second domain comprising a heterologous polypeptide or peptide, wherein the heterologous polypeptide or peptide is not naturally associated with the signal peptide (SP), pectin methyl esterase domain (PED) or catalytic domain (CD).

15

164. A method of increasing thermotolerance or thermostability of a pectate lyase, the method comprising glycosylating a pectate lyase, wherein the polypeptide comprises at least thirty contiguous amino acids of a polypeptide as set forth in claim 55, or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24, thereby increasing the thermotolerance or thermostability of the pectate lyase.

20

165. A method for overexpressing a recombinant pectate lyase in a cell comprising expressing a vector comprising a nucleic acid sequence as set forth in claim 1 or claim 24, wherein overexpression is effected by use of a high activity promoter, a dicistronic vector or by gene amplification of the vector.

25

166. A method of making a transgenic plant comprising the following steps:

(a) introducing a heterologous nucleic acid sequence into the cell, wherein the heterologous nucleic sequence comprises a sequence as set forth in claim 1 or claim 24, thereby producing a transformed plant cell;

30

(b) producing a transgenic plant from the transformed cell.

564462009640

167. The method as set forth in claim 166, wherein the step (a) further comprises introducing the heterologous nucleic acid sequence by electroporation or microinjection of plant cell protoplasts.

5 168. The method as set forth in claim 166, wherein the step (a) comprises introducing the heterologous nucleic acid sequence directly to plant tissue by DNA particle bombardment or by using an *Agrobacterium tumefaciens* host.

169. A method of expressing a heterologous nucleic acid sequence in a  
10 plant cell comprising the following steps:

(a) transforming the plant cell with a heterologous nucleic acid sequence operably linked to a promoter, wherein the heterologous nucleic sequence comprises a sequence as set forth in claim 1 or claim 24;

(b) growing the plant under conditions wherein the heterologous nucleic  
15 acids sequence is expressed in the plant cell.

170. A method for hydrolyzing, breaking up or disrupting a pectin- or pectate (polygalacturonic acid)-comprising composition comprising the following steps:

(a) providing a polypeptide having a pectate lyase activity as set forth in  
20 claim 59, or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24;

(b) providing a composition comprising a pectin or a pectate; and

(c) contacting the polypeptide of step (a) with the composition of step (b) under conditions wherein the polypeptide hydrolyzes, breaks up or disrupts the pectin- or pectate-comprising composition.

25

171. The method as set forth in claim 170, wherein the composition comprises a plant cell wall or a bacterial cell wall.

172. The method as set forth in claim 171, wherein the plant is a cotton  
30 plant, a hemp plant or a flax plant.

173. A method for liquefying or removing a pectin or pectate (polygalacturonic acid) from a composition comprising the following steps:

564462009640

(a) providing a polypeptide having a pectate lyase activity as set forth in claim 59, or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24;

(b) providing a composition comprising a pectin or pectate (polygalacturonic acid); and

5 (c) contacting the polypeptide of step (a) with the composition of step (b) under conditions wherein the polypeptide removes or liquefies the pectin or pectate (polygalacturonic acid).

174. A detergent composition comprising a polypeptide as set forth in claim 59, or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24, wherein the polypeptide has a pectate lyase activity.

175. The detergent composition of claim 174, wherein the pectate lyase is a nonsurface-active pectate lyase or a surface-active pectate lyase.

176. The detergent composition of claim 174, wherein the pectate lyase is formulated in a non-aqueous liquid composition, a cast solid, a granular form, a particulate form, a compressed tablet, a gel form, a paste or a slurry form.

20 177. A method for washing an object comprising the following steps:  
(a) providing a composition comprising a polypeptide having a pectate lyase activity as set forth in claim 59, or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24;

(b) providing an object; and  
25 (c) contacting the polypeptide of step (a) and the object of step (b) under conditions wherein the composition can wash the object.

178. A textile or fabric comprising a polypeptide as set forth in claim 59, or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24.

30 179. A method for fiber, thread, textile or fabric scouring comprising the following steps:

(a) providing a polypeptide having a pectate lyase activity as set forth in claim 59, or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24;



564462009640

- (b) providing a fiber, a thread, a textile or a fabric; and
- (c) contacting the polypeptide of step (a) and the textile or fabric of step (b) under conditions wherein the pectate lyase can scour the fiber, thread, textile or fabric.

5                   180. The method of claim 179, wherein pectate lyase is an alkaline active and thermostable pectate lyase.

10                   181. The method of claim 179, further comprising addition of an alkaline and thermostable amylase in the contacting of step (c).

10                   182. The method of claim 179, wherein the desizing and scouring treatments are combined in a single bath.

15                   183. The method of claim 179, further comprising addition of an alkaline and thermostable amylase in the contacting of step (c).

20                   184. The method of claim 179, wherein the desizing or scouring treatments comprise conditions of between about pH 8.5 to pH 10.0 and temperatures of at about 40°C.

20                   185. The method of claim 179, further comprising addition of a bleaching step.

25                   186. The method of claim 185, wherein the desizing, scouring and bleaching treatments are done simultaneously or sequentially in a single-bath container.

30                   187. The method of claim 185, wherein the bleaching treatment comprises hydrogen peroxide or at least one peroxy compound which can generate hydrogen peroxide when dissolved in water, or combinations thereof, and at least one bleach activator.

                  188. The method of claim 179, wherein the fiber, thread, textile or fabric comprises a cellulosic material.

564462009640

189. The method of claim 188, wherein cellulosic material comprises a crude fiber, a yarn, a woven or knit textile, a cotton, a linen, a flax, a ramie, a rayon, a hemp, a jute or a blend of natural or synthetic fibers.

5 190. A feed or a food comprising a polypeptide as set forth in claim 59, or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24.

191. A method improving the extraction of oil from an oil-rich plant material comprising the following steps:

- 10 (a) providing a polypeptide having a pectate lyase activity as set forth in claim 59, or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24;
- (b) providing an oil-rich plant material; and
- (c) contacting the polypeptide of step (a) and the oil-rich plant material.

15 192. The method of claim 191, wherein the oil-rich plant material comprises an oil-rich seed.

193. The method of claim 191, wherein the oil is a soybean oil, an olive oil, a rapeseed (canola) oil or a sunflower oil.

20

194. A method for preparing a fruit or vegetable juice, syrup, puree or extract comprising the following steps:

- (a) providing a polypeptide having a pectate lyase activity as set forth in claim 59, or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24;
- 25 (b) providing a composition or a liquid comprising a fruit or vegetable material; and
- (c) contacting the polypeptide of step (a) and the composition, thereby preparing the fruit or vegetable juice, syrup, puree or extract.

30 195. A paper or paper product or paper pulp comprising a pectate lyase as set forth in claim 59, or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24.

564462009640

196. A method for treating a paper or a paper or wood pulp comprising the following steps:

(a) providing a polypeptide having a pectate lyase activity as set forth in claim 59, or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24;

5 (b) providing a composition comprising a paper or a paper or wood pulp; and

(c) contacting the polypeptide of step (a) and the composition of step (b) under conditions wherein the pectate lyase can treat the paper or paper or wood pulp.

10 197. A pharmaceutical composition comprising a polypeptide as set forth in claim 59, or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24.

15 198. The pharmaceutical composition of claim 197, wherein the pharmaceutical composition acts as a digestive aid.

199. An oral care product comprising a polypeptide as set forth in claim 59, or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24.

20 200. The oral care product of claim 199, wherein the product comprises a toothpaste, a dental cream, a gel or a tooth powder, an odontic, a mouth wash, a pre- or post brushing rinse formulation, a chewing gum, a lozenge or a candy.

25 201. A method for ameliorating soft-rot spoilage in a plant or plant part comprising administering a composition that decreases the expression or activity of a pectate lyase in the plant or plant part, wherein the composition comprises an antibody as set forth in claim 95, or an antisense oligonucleotide, a ribozyme or an RNAi comprising an antisense oligonucleotide comprising a nucleic acid sequence complementary to or capable of hybridizing under stringent conditions to a sequence as set forth in claim 1 or  
30 claim 24, or a subsequence thereof.

202. The methods of claim 201, wherein the composition is sprayed onto the plant or plant part.

564462009640

203. A method for slowing the normal growth of the powdery mildew pathogen *Erysiphe cichoracearum* in a plant or plant part comprising administering a composition that decreases the expression or activity of a pectate lyase in the plant or plant part, wherein the composition comprises an antibody as set forth in claim 95, or an  
5 antisense oligonucleotide, a ribozyme or an RNAi comprising an antisense oligonucleotide comprising a nucleic acid sequence complementary to or capable of hybridizing under stringent conditions to a sequence as set forth in claim 1 or claim 24, or a subsequence thereof.

10 204. An isolated or recombinant nucleic acid having a sequence comprising a sequence modification of SEQ ID NO:131, wherein the modification of SEQ ID NO:131 comprises one or more of the following changes:

the nucleotides at residues 352 to 354 are CAT or CAC,  
the nucleotides at residues 544 to 546 are GTG, GTT, GTC, or GTA,  
15 the nucleotides at residues 568 to 570 are TTG, TTA, CTT, CTC, CTA, or CTG

the nucleotides at residues 589 to 591 are GGT, GGC, GGA, or GGG,  
the nucleotides at residues 622 to 624 are AAG or AAA,  
the nucleotides at residues 655 to 657 are ATG,  
20 the nucleotides at residues 667 to 669 are GAG or GAA,  
the nucleotides at residues 763 to 765 are CGG, CGT, CGC, CGA, AGA, AGG,

the nucleotides at residues 787 to 789 are AAG or AAA,  
the nucleotides at residues 823 to 825 are TAT or TAC,  
25 the nucleotides at residues 925 to 927 are TGG, or  
the nucleotides at residues 934 to 936 are GTT, GTG, GTC, or GTA.

205. An isolated or recombinant nucleic acid having a sequence comprising a sequence modification of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5,  
30 SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID

564462009640

NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127 or SEQ ID NO:129, wherein the sequence modification comprises one or more of the following changes:

10                   the nucleotides at the equivalent of residues 352 to 354 of SEQ ID NO:131 are changed to CAT or CAC,

                  the nucleotides at the equivalent of residues 544 to 546 of SEQ ID NO:131 are changed to GTG, GTT, GTC, or GTA,

15                   the nucleotides at the equivalent of residues 568 to 570 of SEQ ID NO:131 are changed to TTG, TTA, CTT, CTC, CTA, or CTG

                  the nucleotides at the equivalent of residues 589 to 591 of SEQ ID NO:131 are changed to GGT, GGC, GGA, or GGG,

                  the nucleotides at the equivalent of residues 622 to 624 of SEQ ID NO:131 are changed to AAG or AAA,

20                   the nucleotides at the equivalent of residues 655 to 657 of SEQ ID NO:131 are changed to ATG,

                  the nucleotides at the equivalent of residues 667 to 669 of SEQ ID NO:131 are GAG or GAA,

25                   the nucleotides at the equivalent of residues 763 to 765 of SEQ ID NO:131 are changed to CGG, CGT, CGC, CGA, AGA, AGG,

                  the nucleotides at the equivalent of residues 787 to 789 of SEQ ID NO:131 are changed to AAG or AAA,

                  the nucleotides at the equivalent of residues 823 to 825 of SEQ ID NO:131 are changed to TAT or TAC,

30                   the nucleotides at the equivalent of residues 925 to 927 of SEQ ID NO:131 are changed to TGG, or

                  the nucleotides at the equivalent of residues 934 to 936 of SEQ ID NO:131 are changed to GTT, GTG, GTC, or GTA.

564462009640

206. The isolated or recombinant nucleic acid of claim 204 or claim 205, wherein the nucleic acid encodes a polypeptide having a pectate lyase activity.

207. The isolated or recombinant nucleic acid of claim 206, wherein the  
5 pectate lyase activity of the polypeptide is thermotolerant or thermostable.

208. An isolated or recombinant polypeptide having a sequence comprising a sequence modification of SEQ ID NO:132, wherein the modification of SEQ ID NO:132 comprises one or more of the following mutations: the alanine at amino  
10 acid position 118 is histidine, the alanine at amino acid position 182 is valine, the threonine at amino acid position 190 is leucine, the alanine at amino acid position 197 is glycine, the serine at amino acid position 208 is lysine, the threonine at amino acid position 219 is methionine, the threonine at amino acid position 223 is glutamic acid, the serine at amino acid position 255 is arginine, the serine at amino acid position 263 is  
15 lysine, the asparagine at amino acid position 275 is tyrosine, the tyrosine at amino acid position 309 is tryptophan, or, the serine at amino acid position 312 is valine.

209. An isolated or recombinant polypeptide having a sequence comprising a sequence modification of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6,  
20 SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID  
25 NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:102, SEQ ID NO:104, SEQ ID NO:106, SEQ ID  
30 NO:108, SEQ ID NO:110, SEQ ID NO:112, SEQ ID NO:114, SEQ ID NO:116, SEQ ID NO:118, SEQ ID NO:120, SEQ ID NO:122, SEQ ID NO:124, SEQ ID NO:126, SEQ ID NO:128 or SEQ ID NO:130, wherein the sequence modification comprises one or more of the following changes:

564462009640

the amino acid at the equivalent of the alanine at residue 118 of SEQ ID NO:132 is changed to a histidine,  
the amino acid at the equivalent of the alanine at residue 182 of SEQ ID NO:132 is changed to a valine,  
5 the amino acid at the equivalent of the threonine at residue 190 of SEQ ID NO:132 is changed to a leucine,  
the amino acid at the equivalent of the alanine at residue 197 of SEQ ID NO:132 is changed to a glycine,  
the amino acid at the equivalent of the serine at residue 208 of SEQ ID NO:132 is changed to a lysine,  
10 the amino acid at the equivalent of the threonine at residue 219 of SEQ ID NO:132 is changed to a methionine,  
the amino acid at the equivalent of the threonine at residue 223 of SEQ ID NO:132 is changed to a glutamic acid,  
15 the amino acid at the equivalent of the serine at residue 255 of SEQ ID NO:132 is changed to a arginine,  
the amino acid at the equivalent of the serine at residue 263 of SEQ ID NO:132 is changed to a lysine,  
the amino acid at the equivalent of the asparagine at residue 275 of SEQ ID NO:132 is changed to a tyrosine,  
20 the amino acid at the equivalent of the tyrosine at residue 309 of SEQ ID NO:132 is changed to a tryptophan, or,  
the amino acid at the equivalent of the serine at residue 312 of SEQ ID NO:132 is changed to a valine.

25

210. The isolated or recombinant polypeptide of claim 208 or claim 209, wherein the polypeptide has a pectate lyase activity.

211. The isolated or recombinant polypeptide of claim 210, wherein the  
30 pectate lyase activity of the polypeptide is thermotolerant or thermostable.

212. A method for generating a modified pectate-lyase encoding nucleic acid comprising making one or more sequence modifications to a pectate-lyase encoding

564462009640

nucleic acid, wherein the changes in the pectate-lyase encoding nucleic acid are equivalent to one or more of the following:

- changing nucleotides at the equivalent of residues 352 to 354 of SEQ ID NO:131 to CAT or CAC,
- 5 changing nucleotides at the equivalent of residues 544 to 546 of SEQ ID NO:131 to GTG, GTT, GTC, or GTA,
- changing nucleotides at the equivalent of residues 568 to 570 of SEQ ID NO:131 to TTG, TTA, CTT, CTC, CTA, or CTG
- changing nucleotides at the equivalent of residues 589 to 591 of SEQ ID NO:131 to GGT, GGC, GGA, or GGG,
- 10 changing nucleotides at the equivalent of residues 622 to 624 of SEQ ID NO:131 to AAG or AAA,
- changing nucleotides at the equivalent of residues 655 to 657 of SEQ ID NO:131 to ATG,
- 15 changing nucleotides at the equivalent of residues 667 to 669 of SEQ ID NO:131 to GAG or GAA,
- the nucleotides at the equivalent of residues 763 to 765 of SEQ ID NO:131 to CGG, CGT, CGC, CGA, AGA, AGG,
- changing nucleotides at the equivalent of residues 787 to 789 of SEQ ID NO:131 to AAG or AAA,
- 20 changing nucleotides at the equivalent of residues 823 to 825 of SEQ ID NO:131 to TAT or TAC,
- changing nucleotides at the equivalent of residues 925 to 927 of SEQ ID NO:131 to TGG, or
- 25 changing nucleotides at the equivalent of residues 934 to 936 of SEQ ID NO:131 to GTT, GTG, GTC, or GTA.

213. The method of claim 212, wherein the modified pectate lyase activity has a thermotolerant or thermostable activity.

30

214. The method of claim 212, wherein the pectate-lyase encoding nucleic acid comprises a nucleic acid having a sequence as set forth in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID



564462009640

NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID  
 NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID  
 NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID  
 NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID  
 5 NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID  
 NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID  
 NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID  
 NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID  
 NO:103, SEQ ID NO:105, SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:111, SEQ ID  
 10 NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SEQ ID  
 NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:131 or SEQ  
 ID NO:133.

215. A method for generating a modified pectate lyase comprising  
 15 making one or more sequence modifications to a pectate lyase, wherein the changes in the  
 pectate lyase are equivalent to one or more of the following changes:

the amino acid at the equivalent of the alanine at residue 118 of SEQ ID  
 NO:132 is changed to a histidine,

the amino acid at the equivalent of the alanine at residue 182 of SEQ ID  
 20 NO:132 is changed to a valine,

the amino acid at the equivalent of the threonine at residue 190 of SEQ ID  
 NO:132 is changed to a leucine,

the amino acid at the equivalent of the alanine at residue 197 of SEQ ID  
 NO:132 is changed to a glycine,

the amino acid at the equivalent of the serine at residue 208 of SEQ ID  
 25 NO:132 is changed to a lysine,

the amino acid at the equivalent of the threonine at residue 219 of SEQ ID  
 NO:132 is changed to a methionine,

the amino acid at the equivalent of the threonine at residue 223 of SEQ ID  
 30 NO:132 is changed to a glutamic acid,

the amino acid at the equivalent of the serine at residue 255 of SEQ ID  
 NO:132 is changed to an arginine,

the amino acid at the equivalent of the serine at residue 263 of SEQ ID  
 NO:132 is changed to a lysine,

564462009640

the amino acid at the equivalent of the asparagine at residue 275 of SEQ ID NO:132 is changed to a tyrosine,

the amino acid at the equivalent of the tyrosine at residue 309 of SEQ ID NO:132 is changed to a tryptophan, or,

5 the amino acid at the equivalent of the serine at residue 312 of SEQ ID NO:132 is changed to a valine.

216. The method of claim 215, wherein the pectate lyase comprises a sequence as set forth in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:102, SEQ ID NO:104, SEQ ID NO:106, SEQ ID NO:108, SEQ ID NO:110, SEQ ID NO:112, SEQ ID NO:114, SEQ ID NO:116, SEQ ID NO:118, SEQ ID NO:120, SEQ ID NO:122, SEQ ID NO:124, SEQ ID NO:126, SEQ ID NO:128 or SEQ ID NO:130.

217. The method of claim 216, wherein the modified pectate lyase activity has a thermotolerant or thermostable activity.

218. A formulation for treating a material with a pectate lyase comprising a pectate lyase as set forth in claim 59, wherein the formulation comprises a dosage of pectate lyase in the range of between about 1 gram per ton treated material and 100 or more grams per ton per ton treated material.

219. The formulation of claim 218, wherein the dosage is between about 10 grams per ton and 90 grams per ton, between about 20 grams per ton and 80 gram per

564462009640

ton, between about 30 grams per ton and 70 grams per ton, or between about 40 grams per ton and 50 grams per ton.

220. The formulation of claim 218, wherein the dosage is between  
5 about 1  $\mu\text{g}$  per gram and 100 or more  $\mu\text{g}$  per gram, between about 10  $\mu\text{g}$  per gram and 90  $\mu\text{g}$  per gram, between about 20  $\mu\text{g}$  per gram and 80  $\mu\text{g}$  per gram, between about 30  $\mu\text{g}$  per gram and 70  $\mu\text{g}$  per gram, or between about 40  $\mu\text{g}$  per gram and 50  $\mu\text{g}$  per gram.

221. The formulation of claim 218, wherein the dosage is between about  
10 0.5 mg per pound and 50 or more mg per pound, between about 1 mg per pound and 45 mg per pound, between about 5 mg per pound and 40 mg per pound, between about 10 mg per pound and 35 mg per pound or between about 15 mg per pound and 30 mg per pound.

222. The formulation of claim 218, wherein the formulation comprises a  
15 fabric or a cloth.

223. The formulation of claim 218, wherein the formulation is a water-  
based formulation.

20 224. The formulation of claim 223, wherein the dosage comprises an enzyme strength of between about 500 to 30,000 units/ml.

225. The formulation of claim 224, wherein the dosage comprises an  
25 enzyme strength of between about 1000 to 25,000 units/ml, 1000 to 20,000 units/ml, 1000 to 15000 units/ml, 1000 to 10,000 units/ml, between about 1000 to 5000 units/ml, between about 2000 to 20000 units/ml, between about 2000 to 10000 units/ml, or between about 2000 to 5000 units/ml.

30 226. The formulation of claim 225, wherein the dosage comprises an enzyme strength of about 1000 u/ml.

227. The formulation of claim 218, wherein the formulation comprises a lyophilized enzyme.

564462009640

228. The formulation of claim 218, wherein the formulation comprises a lyophilized enzyme resuspended in water.

5 229. The formulation of claim 218, further comprising a glycerol, sucrose, sodium chloride, dextrin, propylene glycol, sorbitol, sodium sulphate or TRIS, or an equivalent.

230. The formulation of claim 218, further comprising a buffer.

10

231. The formulation of claim 218, wherein the buffer comprises pH 7, 35% glycerol, 0.1% sodium benzoate, 0.1% potassium sorbate; pH 7, 35% glycerol, 300 ppm proxel; pH 7, 10% sodium chloride, 25% glycerol, 0.1% sodium benzoate, 0.1% potassium sorbate; pH 7, 10% sodium chloride, 25% glycerol, 300 ppm proxel; pH 5.5, 35% glycerol, 0.1% sodium benzoate, 0.1% potassium sorbate; pH 5.5, 35% glycerol, 300 ppm proxel; pH 5.5, 10% sodium chloride, 25% glycerol, 0.1% sodium benzoate, 0.1% potassium sorbate; or, 20mM acetate buffer, pH 5.5, 35% glycerol; 20 mM MOPS, pH 7 or 25 mM MOPS, 50 mM NaCl, pH 7.5; pH 5.0, 40mM TRIS; pH 7.0, 40mM TRIS; pH 8.0, 40mM TRIS; pH 7.5, 50% glycerol; pH 7.5, 20% NaCl; pH 7.5, 30% propylene glycol; pH 7.5, 100mM sodium sulfate; pH 5.5, 35% glycerol; or, any combination thereof, or, equivalents thereof.

15 20

232. A bioscouring process comprising the following steps:

- (a) providing a pectate lyase as set forth in claim 57;
- 25 (b) providing a pectin- or polygalacturonic acid- comprising material;
- (c) contacting the pectate lyase of (a) with the material of (b) under conditions comprising about pH 8.5, in bicarbonate buffer, comprising a non-ionic wetting agent at, about 1 g/L, where the pectate lyase ratio in an enzyme bath is between about 10:1 to 50:1 L pectate lyase:kg of material, where the pectate lyase dose is between about 0.1 and 0.2 ml of a concentrated extract per kg of material, or equivalent, at a
- 30 temperature range between about 50°C to 70°C, and a treatment time about 20 min.

233. The bioscouring process of claim 232, wherein the material comprises a fabric or a cloth.

564462009640

234. The bioscouring process of claim 232, wherein the pectate lyase dose is about 0.137 ml of a concentrated extract per kg of material, or equivalent.

- 5                    235. The bioscouring process of claim 232, wherein the contacting step further comprises use of a chelant, wherein the chelant is excluded from the enzyme bath and is added after about 20 minutes of enzyme treatment and retained for about 10 minutes before discharging bath.